IBACON

Institut für
Biologische Analytik und
Consulting IBACON GmbH

Final Report

Acute Toxicity of Wacker BS 1701 to *Daphnia magna* in a 48-hour Immobilization Test

(GLP compliant study based on the Directive 92/69/EEC, C.2, 1992 and the OECD No. 202, Part I, 1984)

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Study Completion Date: March 08, 2001

Sponsor

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Johannes-Hess-Straße 24
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Test Facility

Institut für Biologische Analytik
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Project 9541220

HESSISCHES MINISTERIUM
FÜR UMWELT, LANDWIRTSCHAFT
UND FORSTEN

GLP-Bescheinigung

Bescheinigung

Hiermit wird bestätigt, daß die Prüfeinrichtung
Institut für Biologische Analytik
in 64380 Roßdorf
Industriestraße 1
(Ort, Anschrift)
der IBACON
(Firma)
am 08. und 09 Dezember 1998
(Datum)

von der für die Überwachung zuständigen Behörden über die Einhaltung der Grundsätze der Guten Laborpraxis inspiziert worden ist.

Es wird hiermit bestätigt, daß folgende Prüfungen in dieser Prüfeinrichtung nach den Grundsätzen der Guten Laborpraxis durchgeführt werden:

Prüfungen zur Bestimmung der physikalisch-chemischen Eigenschaften und Gehaltsbestimmungen
Ökotoxikologische Prüfungen zur Bestimmung der Auswirkungen auf aquatische und terrestrische Organismen
Prüfungen zum Verhalten im Boden, im Wasser und in der Luft, Prüfungen zur Bioakkumulation und zur Metabolisierung
Prüfungen zur Bestimmung von Rückständen

Im Auftrag

(Dr. Hecker) Wiesbaden, den 20. August 1999

Certificate

It is hereby certified that the test facility
Insitut für Biologische Analytik
in 64380 Roßdorf
Industriestraße 1
(location, address)
of IBACON
(company name)
on 08. und 09. Dezember 1998
(date)

was inspected by the competent authority regarding compliance with the Principles of Good Laboratory Practice.

It is hereby certified that studies in this test facility are conducted in compliance with the Principles of Good Laboratory Practice:

Physical and chemical properties
and determination of content
Environmental toxicity studies
on aquatic and terrestrial organisms
Behaviour in water soil and air,
Bioaccumulation and metabolism
Residues

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1. Summary

Title: Acute Toxicity of Wacker BS 1701 to *Daphnia magna* in a 48-hour Immobilization Test

Guidelines/Recommendations:

- Commission Directive 92/69/EEC, Annex Part C, C.2: "Acute Toxicity for *Daphnia*", Official Journal of the European Communities No. L 383 A, dated December 29, 1992.
- OECD Guideline for Testing of Chemicals, Section 2, No. 202: "*Daphnia sp.*, Acute Immobilisation Test and Reproduction Test", Part I, adopted April 04, 1984.

Purpose: The influence of the test item Wacker BS 1701 on the mobility respectively survival of *Daphnia magna* was tested. Young *Daphnia* were exposed in a semi static test to the test item for 48 hours, added to water at a range of concentrations.

Test Concentrations: Nominal 0.32, 1.0, 3.2, 10, 32 and 100 mg test item/L, and a control. Since the test item is not well soluble in test water, additionally a filtrate of a supersaturated stock suspension of nominal 100 mg/L was tested to determine the toxic effect of the dissolved part of the test item only and to avoid effects due to undissolved test item.

Analytical Results: The analytical dose verification, based on the analysis of silicon by means of ICP-AES (Inductive Couples Plasma Emission-Spectrometry) and graphite oven AAS was not possible. The results of the ICP-AES analysis did not give reproducible and acceptable results since in the atomizing chamber the appearing phase separated to an organic and a water phase, resulting in memory effects. Typical standard deviation of repeated analysis was 91%. The graphite oven AAS analysis failed due to formation of silicon carbide in the graphite tube, resulting in memory effects. Typical recovery was in the range from14 to 181%. Since no verification is practicable with usual analytical methods no analytical dose verification could be done in the present test. Therefore, all biological results are related to nominal concentrations of the test item.

Biological Results:

24-hour EC 50:	26.8 mg test item/L
95 % confidence value:	19.4 – 37.1 mg test item/L
24-hour EC 0:	10 mg test item/L
24-hour EC 100:	100 mg test item/L
48-hour EC 50:	12.0 mg test item/L
95 % confidence value:	8.7 – 16.6 mg test item/L
48-hour NOEC and EC 0:	3.2 mg test item/L
48-hour EC 100	32 mg test item/L

In the filtrate of the supersaturated stock suspension no toxic effect was determined after 24 hours test duration. After 48 hours an immobilization rate of 80 % was determined. This toxic effect was caused only by dissolved test item, respectively hydrolysis products of the test item.

2. Survey of the Study

2.1 General Information

Title:	Acute Toxicity of Wacker BS 1701 to *Daphnia magna* in a 48-hour Immobilization Test
Sponsor:	Wacker-Chemie GmbH Werk Burghausen Johannes-Hess-Straße 24 84489 Burghausen Germany
Monitoring:	Dr. Axel Bosch
Test Item:	Wacker BS 1701
Test Facility:	Institut für Biologische Analytik und Consulting IBACON GmbH Arheilger Weg 17 64380 Rossdorf Germany
IBACON-Project:	9541220

Project Staff:

Test Facility Management:	Dr. Ralf Petto
Study Director:	Dr. Johannes Hertl
Principal Investigator:	Dr. Kiefer, IFU Umweltanalytik GmbH
Performing Laboratory for Analytical Part:	Arbeitsgemeinschaft GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH Eutinger Str. 24 75223 Niefern-Öschelbronn
Technical Coordination:	Heidi Breitwieser
Head of Quality Assurance Unit (QAU):	Dipl. Biol. Christiane Rutschmann-Fröhlich
Quality Assurance Unit Managers:	Dipl. Biol. Antje Pfützner Dipl. Biol. Erika Schnellbächer

Schedule:

Study Initiation Date:	December 07, 2000
Date of 1st Study Protocol Amendment:	February 28, 2001
Experimental Starting Date:	January 09, 2001
Experimental Completion Date:	January 11, 2001
Draft Report Date:	February 06, 2001
Study Completion Date:	March 08, 2001

2.2 Good Laboratory Practice

This study was performed in compliance with:

- The OECD Principles of Good Laboratory Practice (as revised in 1997) and the
- Chemikaliengesetz (*'Chemicals Act'*) der Bundesrepublik Deutschland (ChemG), Anhang 1 (*'Annex 1'*), 1994/97.

Quality Assurance of the study was the responsibility of the test facilities (i.e. IBACON GmbH and IFU Umweltanalytik GmbH) and was carried out in accordance with the GLP regulations and SOPs.
This study and/or procedures was periodically inspected by the Quality Assurance Unit (QAU) of the respective test facility and the dates and phases of the inspections were included into the final report. The data contained with the final report were audited in comparison to the raw data. A quality assurance statement, signed by the Quality Assurance Unit, is included into the final report.

2.3 Archiving

The following data / sample(s) will be archived

for 15 years:
- the study protocol
- the study protocol amendment
- one certified copy of the final report

for at least 2 years: one sample of the test item

following the date on which the final report is audited by the Quality Assurance Unit at:

Institut für Biologische Analytik
und Consulting IBACON GmbH
Arheilger Weg 17
D-64380 Rossdorf
Germany

For the period demanded by the principles of GLP, samples of the test item and the reference item and all raw data of the analytical part will be stored in the archives of the performing laboratory Arbeitsgemeinschaft GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH. No raw data or material relating to the study will be discarded without the sponsor's prior consent.

2.4 Signatures

Study Director: Dr. Johannes Hertl

date: March 08, 2001

Test Facility Management: Dr. Ralf Petto

date: March 08, 2001

3. Quality Assurance Unit Statement

Test Facility:	Institut für Biologische Analytik und Consulting IBACON GmbH Arheilger Weg 17 64380 Rossdorf Germany
IBACON Project:	9541220
Title of the Study:	Acute Toxicity of Wacker BS 1701 to *Daphnia magna* in a 48-hour Immobilization Test
Test Item:	Wacker BS 1701
Study Director:	Dr. Johannes Hertl

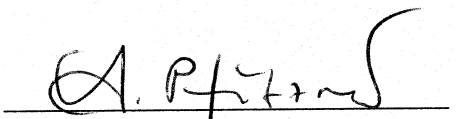
The pre-experiments as mentioned in the final report were not inspected.

Study based Inspections

Phases inspected	Dates of QAU Inspections	Dates of Reports to Study Director and to Test Facility Management
Study Protocol	December 07, 2000	December 07, 2000
1st Study Protocol Amendment	February 26, 2001	February 26, 2001
Experimental Phase	January 09 – 11,2001	January 11, 2001
Draft Report	February 26 – 27, 2001	February 27, 2001
Final Report	March 08, 2001	March 08, 2001

This statement confirms that the final report reflects the raw data.

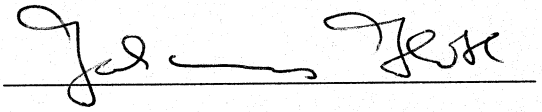
Quality Assurance Unit: **Dipl. Biol. Antje Pfützner**



date: March 09, 2001

4. Statement of Compliance

IBACON Project:	9541220
Title of the Study:	Acute Toxicity of Wacker BS 1701 to *Daphnia magna* in a 48-hour Immobilization Test
Test Item:	Wacker BS 1701
Study Director:	Dr. Johannes Hertl
GLP-Regulations:	• The OECD Principles of Good Laboratory Practice (as revised in 1997) and the • Chemikaliengesetz (*'Chemicals Act'*) der Bundesrepublik Deutschland (ChemG), Anhang 1 *('Annex 1')*, 1994/97.
Integrity of the Study:	This study (excluding pre-tests), performed in the test facility of IBACON, was conducted in compliance with the Good Laboratory Practice regulations. There were no circumstances that may have affected the quality or integrity of the study. The analytical dose verification was performed at IFU Umweltanalytik GmbH, Pforzheim.
Study Director:	Dr. Johannes Hertl



date: Nov 05, 2001

5. Objectives of the Study

5.1 Title

Acute Toxicity of Wacker BS 1701 to *Daphnia magna* in a 48-hour Immobilization Test

5.2 Purpose

The purpose of this study was to evaluate the influence of the test item Wacker BS 1701 on the mobility respectively survival of *Daphnia magna*. Young *Daphnia* were exposed in a semi static test to the test item for 48 hours, added to water at a range of concentrations. Under otherwise identical test conditions, different concentrations of the test item result in different percentages of *Daphnia* being no longer capable of swimming at the end of the test or being dead.

Due to the low solubility of the test item in test water, additionally a filtrate of a supersaturated stock suspension of nominal 100 mg/L was tested to determine the toxic effect of the dissolved part of the test item only and to avoid effects due to undissolved test item.

The test method of application and the test species *Daphnia magna* are recommended by the test guidelines.

5.3 Guidelines / Recommendations

This study was designed to comply with the following methods:

- Commission Directive 92/69/EEC, Annex Part C, C.2: "Acute Toxicity for *Daphnia*", Official Journal of the European Communities No. L 383 A, dated December 29, 1992.
- OECD Guideline for Testing of Chemicals, Section 2, No. 202: "*Daphnia sp.*, Acute Immobilisation Test and Reproduction Test", Part I, adopted April 04, 1984.

6. Material and Methods

6.1 Test Item and Control

Test Item

The test item and the information concerning the test item were provided by the sponsor.

Name:	Wacker BS 1701
Batch No.:	KH 02343
Active Ingredient(s) / Purity:	alkylalkoxysilane / 98.53 %, (GC)
Certificate of Analysis / Date:	20.07.2000
Aggregate State at RT:	liquid
Molecular Weight:	276.49 g/mol
Molecular Formula:	$C_{14}H_{32}O_3Si$
Colour:	colourless
Density (at 25 °C):	0.86 g/cm^3
Solubility:	in water: insoluble
Stability:	pure: see expiry date in water: not indicated by the sponsor
Expiry Date:	November 2001
Storage:	in original container, at room temperature, in the dark

Control

Control:	reconstituted water (see 6.5)

6.2 Test Organism

Species:	*Daphnia magna* (Straus), clone 5
Age at Test Start:	8 – 23.5 hours old
Sex:	female
Origin:	supplied 1997 by the Umweltbundesamt, Institut für Wasser-, Boden- und Lufthygiene, Berlin, Germany
Breeding Conditions:	The *Daphnia* were bred in the laboratories of IBACON under similar temperature and light conditions as in the test, and in reconstituted water of a similar quality regarding to pH, components of the main ions and total hardness as the test water used in the test (see below). The test organisms were not first brood progeny.
Toxic Standard:	For the evaluation of the quality of the *Daphnia* clone and the experimental conditions the substance potassium dichromate p.A. (E. Merck, Darmstadt, Germany) is tested at least twice a year to demonstrate satisfactory test conditions.
Acclimatisation:	for 8 hours under test conditions (see 6.4)

6.3 Test Units

Type and Size:	glass beakers of 250 mL volume with 150 mL test medium
Identification:	Each test unit was uniquely identified with study number, treatment and replicate number.

6.4 Test Conditions

Surrounding Type:	controlled environment room
Water Temperature:	21 °C
Light Regime:	16 h light : 8 h dark
Light Intensity:	370 - 380 lux

6.5 Test Water

Reconstituted Water: In deionized water (conductivity < 5 μScm^{-1}) analytical grade salts were added to following nominal concentrations:

$CaCl_2 \times 2H_2O$	2.0 mmol/L	(= 294.0 mg/L)
$MgSO_4 \times 7H_2O$	0.5 mmol/L	(= 123.0 mg/L)
$NaHCO_3$	0.75 mmol/L	(= 65.0 mg/L)
KCl	0.075 mmol/L	(= 5.8 mg/L)

Water Hardness : 2.5 mmol/L (= 250.0 mg/L) as $CaCO_3$
Alkalinity : 0.8 mmol/L

ratio of Ca : Mg = 4 : 1 (based on molarity)
Na : K = 10 : 1 (based on molarity)

6.6 Application of the Test Item and the Control

Pre-Experiments:	Pre-experiments were carried out to determine the solubility of the test item in test water. The test item could only in small quantities be dissolved in test water. These pre-experiments to the solubility of the test item were not performed in compliance with GLP-Regulations, but the raw data of these pre-experiments will be archived under the project number of the present study.
Range-Finding Test:	The test concentrations were based on the results of range-finding tests. The range-finding tests were not performed in compliance with GLP-Regulations and are excluded from the statement of compliance, but the raw data of these range-finding tests will be archived under the project number of the present study.
Test Concentrations:	The nominal concentrations 0.32, 1.0, 3.2, 10, 32 and 100 mg test item/L were tested. Additionally, a control was tested in parallel (test water without addition of the test item). The enlarged spacing factor of 3.2 between the test concentrations was chosen, because according to the results of the range-finding tests the concentration-effect relationship was rather flat and thus a large concentration range had to be tested. Due to the low solubility of the test item in test water, additionally a filtrate of a supersaturated stock suspension of nominal 100 mg/L was tested to determine the toxic effect of the dissolved part of the test item only and to avoid effects due to undissolved test item.
Dosage of Test Item:	The test medium of the highest test concentration of nominal 100 mg/L was prepared by suspending 150 mg test item into 1.5 litre test water by intense stirring for 30 minutes and short ultrasonic treatment for 15 minutes. Adequate volumes were taken from the stirred test medium and were diluted with test water to prepare the test media of all other test concentrations. The filtrate of the supersaturated stock suspension was prepared as follows: a stock suspension of 100 mg/L was ultrasonically treated for 10 minutes and stirred for 24 hours to dissolve the highest possible rate of test item. Then the stock suspension was filtered through a cellulose nitrate filter (pore size 0.45 µm). This filtrate was used as test medium.

6.7 Course of the Test

Test Procedure:	A semi static test procedure was chosen to keep the concentrations of the parent compound Wacker BS 1701 in the test medium as constant as possible during the test period. Therefore, all test media were renewed after 24 hours test duration.
Introduction of Individuals:	20 *Daphnia* per control and test concentration, divided into two groups of ten animals, each group in 150 mL test medium
Exposure Time:	48 hours

6.8 Test Parameters

Mortality:

The immobility or mortality of the *Daphnia* was determined by visual controls after 24 and 48 hours. Those animals not able to swim within 15 seconds after gentle agitation of the test beaker were considered to be immobile.

Measurement of pH, dissolved Oxygen and Water Temperature:

At test start, after 24 hours and at test end the pH-values and the dissolved oxygen concentrations were determined in the freshly prepared and old test media of all test concentrations and the control. At the same times the water temperature was determined in the test medium of one control beaker.

Behaviour of the Test Item in Test Water:

The behaviour of the test item in test water was determined at the start of the test, after 24 and 48 hours test duration in all test concentrations.

6.9 Result Evaluation

Definitions:

NOEC (48 h):	No Observed Effect Concentration: highest test concentration at which no significant effect was determined after 48 hours test duration
EC 50:	the calculated concentration of the test item which results in a 50 % immobilization rate
EC 0:	highest test concentration without a significant number of immobilized test animals
EC 100:	the lowest test concentration at which all test animals are immobile

Statistical Analysis:

The 24-hour and 48-hour EC 50 and the 95 % confidence limits were calculated by the moving average interpolation. The NOEC, EC 0 and EC 100 were determined directly from the raw data.

6.10 Analysis of the Test Item Concentrations

Sampling:

Duplicate samples from the freshly prepared test media of all test concentrations, the filtrate and the control were taken at the start of the test and on day one of exposure.

For the determination of the stability of the test item under the test conditions, respectively the maintenance of the test item concentrations during the test period, duplicate samples from the test media of all test concentrations, the filtrate and the control were collected after 24 hours and at the end of the test (after 48 hours).

Storage of the Samples:

All samples were stored in a refrigerator immediately after sampling and were kept stored to enable additional analyses. After delivery of the final report all samples will be discarded.

Analyses:	The analytical verification was performed in the laboratories of IFU Umweltanalytik GmbH, Bleichstraße 19, 75173 Pforzheim, Germany under the project number 20011012/01-UW. Since no analytical dose verification was practicable with usual analytical methods no analytical dose verification could be done in the present test.
Analytical Method:	The analytical dose verification, based on the analysis of silicon was tried by means of ICP-AES (Inductive Couples Plasma Emission-Spectrometry) and graphite oven AAS. ICP Spectroflame D, Spectro Analytical Systems, Perkin Elmer AAS 4100 with HGA 700

6.11 Validity Criteria of the Study

Control:	The experiment is valid because no *Daphnia* died in the control and no *Daphnia* were trapped at the water surface.
Dissolved Oxygen Concentration:	At the end of the test the dissolved oxygen concentration in the test media was > 60 % of the air saturation value at the temperature used.

6.12 Deviations to the Study Protocol

There were no deviations to the study protocol.

7. Results and Discussion

7.1 Analytical Results

Results of the ICP-AES Analysis:

At the beginning of the measuring sequence, a calibration curve was prepared. Five concentrations (blank, 2.0 mg/L, 4.0 mg/L, 8.0 mg/L and 16 mg/L) of the reference item covering the concentration range of the test item solutions were injected.

The analysis of the test item solutions to determine the repeatability and the recovery of the analytical method, respectively, did not give reproducable and acceptable results. Typical standard deviation of repeated analysis was 91%.

The reason for this observation was the appearing phase separation of the emulsion. In the atomizing chamber of the ICP-AES, there appeared an organic and a water phase, resulting in memory effects in the course of the measurements.

The alternate sample preparation by dissolving the test item in acetone, ethyl acetate, or ethanol, respectively, and the technical equipment variation using a cross flow atomizer did not give better results.

Results of the Graphite Furnace AAS-Analysis:

At the beginning of the measuring sequence, a calibration curve was prepared. Six concentrations of the reference item (blank, 100 µg/L, 200 µg/L, 300 µg/L, 400 µg/L and 500 µg/L) covering the concentration range of the test item solutions were injected.

The analysis of the test item solutions to determine the repeatability and the recovery of the analytical method, respectively, did not give reproducable and acceptable results. Typical recovery was in the range from14 to 181%.

The reason for this observation was the was the formation of silicon carbide in the graphite tube, resulting in memory effects in the course of the measurements.

Concentrations of Wacker BS 1701 in the Test Media:

Considering the results of this study, it was not possible to establish a reproducable method to determine the concentrations of Wacker BS 1701 (nominal, stability and homogenity) by either ICP-AES or graphite furnace AAS. However, no suitable quantification method for the test item from aqueous solution was available.

According to RCC project 797242 the water solubility of the test item was determined to be < 0.25 mg/L. In the study no additional formation of the hydrolysis product ethanol was determined and the test item is stated to be stable in water. However, no suitable quantification method for the test item from aqueous solution was available. Only a hydrolysis product was determined. A hy-

drolysis study could not be performed due to the special properties of the test item.
For the performance of the aquatic tests the test item was ultrasonically treated to dissolve as much test item as possible in test water. Due to a higher surface of the test item and an accelerated reaction with water, this treatment may lead to a higher concentration of hydrolysis product than treatment by stirring only. To achieve the highest concentration of test item which could be dissolved in test water, this procedure was chosen, although these conditions never appear in natural environment. Dissolved test item may be a mixture of parent compound and hydrolysis products.
Since no verification is practicable with usual analytical methods no analytical dose verification could be done in the present test. Therefore, all reported results are related to nominal concentrations of the test item.

7.2 Biological Results

Pre-Experiments:

In a pre-experiment, without GLP, the nominal test concentrations of 0.1, 1.0, 10 and 100 mg Wacker BS 1701/L were tested by dosing adequate amounts of an intensely stirred stock suspension of 100 mg/L into each test beaker. A static test procedure was used. At the highest test concentration of nominal 100 mg/L all *Daphnia* were dead within 48 hours. At the nominal concentration of 10 mg/L 30 % immobility and at the test concentration of nominal 1.0 mg/L 20 % immobility was observed. At the lowest test concentration of nominal 0.1 mg/L and in the control no mortality or immobilization was determined.

Additionally, a filtrate of a supersaturated stock suspension of 100 mg/L, stirred for 22 hours and filtered through a cellulose-nitrate filter (pore size 0.45 µm) and the dilution 1:10 of the filtrate were tested in a static test system. In the filtrate and the control no immobility was determined and in the dilution 1:10 of the filtrate only one *Daphnia* (10 %) was immobile after 48 hours test duration.

Main Test:

To keep the concentrations of dissolved test item as constant as possible in water a semi static test procedure was chosen.

Signs of Intoxication after 24 hours:

After 24 hours of exposure in the control and in the test concentrations up to and including nominal 10 mg/L no mortality or immobility of the test animals was observed. However, at the concentration of nominal 10 mg/L all *Daphnia* were trapped at the water surface. At the concentration of nominal 32 mg/L an immobilization rate of 65 % was determined, all surviving *Daphnia* were trapped at the water surface but were not immobile. At the concentration of nominal 100 mg/L all *Daphnia* were immobile after 24 hours test duration.

Results after 24 hours:	The 24-hour EC 50 of the test item was calculated to be 26.8 mg test item/L with 95 % confidence limits from 19.4 to 37.1 mg/L. The 24-hour EC 0 was nominal 10 mg test item/L, the 24-hour EC 100 amounted to nominal 100 mg test item/L. In the filtrate of the stock suspension of 100 mg/L no immobilization was determined, but all *Daphnia* were trapped at the water surface.
Signs of Intoxication after 48 hours:	After 48 hours of exposure the toxicity of the test item to *Daphnia magna* had increased. In the control and in the test concentrations of nominal 0.32 and 3.2 mg test item/L no mortality or other signs of intoxication were observed. At the test concentration of nominal 1.0 mg/L, an immobilization rate of 10 % was determined. However this immobilization rate was not estimated as a significant toxic effect, because according to the test guidelines this immobilization rate is also tolerated in the control, and additionally at the next higher test concentration of nominal 3.2 mg test item/L no immobilization and no other signs of intoxication were observed. At the test concentration of nominal 10 mg/L an immobilization rate of 35 % was determined and all other *Daphnia* were trapped at the water surface. At the two highest test concentrations of nominal 32 and 100 mg/L all *Daphnia* were immobile after 48 hours.
Results after 48 hours:	The 48-hour EC 50 was calculated to be 12.0 mg test item/L with 95 % confidence limits from 8.7 to 16.6 mg/L. The 48-hour EC 0 and the 48-hour NOEC (highest concentration tested without toxic effects after 48 hours) of Wacker BS 1701 were determined to be nominal 3.2 mg test item/L, since no significant immobilization rate and no other signs of intoxication were observed at the test animals up to and including this test concentration. The 48-hour EC 100 amounted to nominal 32 mg test item/L. In the filtrate of the stock suspension of 100 mg/L an immobilization rate of 80 % was determined and all surviving *Daphnia* were trapped at the water surface.
Comparison between Pre-Experiments and Main Test:	In the pre-experiments and in the main test, where the test item was dosed into the test beakers by using a stock suspension the results after 48 hours are nearly the same. Although in the pre-experiment a static test system and in the main test a semi static test system were chosen, the toxicity of the test item was identical. In both tests the main part of the test item was not dissolved. A lost part of dissolved test item, respectively hydrolysis products, possible due to adsorption, can be renewed from the undissolved test item which is available in the test system.

In both experiments the toxic effects may be caused by dissolved test item, respectively hydrolysis products and physical effects of undissolved test item on the animals.

In the filtrates the concentration of dissolved test item, respectively hydrolysis products may be higher than the solubility limit, stated in RCC report 797242, due to the ultrasonic treatment, which may cause a faster hydrolysis.

The effects in the main test are supposed to be caused by dissolved test item and or the hydrolysis products. No undissolved test item was present in water.

In the static pre experiment, using a filtrate, no toxic effect was determined after 48 hours test duration. In the semi static main test an immobilization of *Daphnia* was determined after 48 hours. The test medium renewal causes a higher toxic effect. Therefore, it can be supposed that during the test a loss of dissolved test item and hydrolysis products (may be due to adsorption) occurred.

7.3 pH, dissolved Oxygen Concentrations, Water Temperature and Behaviour of the Test Item in Test Water

pH-Values:	pH 7.6 to 7.9 (see Table 3)
Dissolved Oxygen Concentrations:	at least 7.9 mg/L or higher (see Table 2)
Water Temperature:	21 °C
Behaviour of the Test Item:	No remarkable observations were made at the nominal test concentrations of 0.32 to 10 mg/L and in the filtrate. At the nominal concentrations of 32 and 100 mg test item/L a part of the test item was swimming at the surface of the test water.

8. References

1. Chemikaliengesetz der Bundesrepublik Deutschland (ChemG), Anhang 1, in der Fassung der Bekanntmachung vom 25. Juli 1994 (BGBl. I S. 1703) mit Änderungen vom 27. September 1994 (BGBl. I S. 2705) und 14. Mai 1997 (BGBl I S. 1060)

2. Commission Directive 92/69/EEC, Annex Part C, C.2: „Acute Toxicity for Daphnia", Official Journal of the European Communities No. L 383 A, dated December 29, 1992

3. Finney, D.J. (1978): Statistical Methods in Biological Assay, 3rd Edition, Charles Griffin, London

4. OECD Guideline for Testing of Chemicals, Section 2, No. 202: "*Daphnia sp.*, Acute Immobilisation Test and Reproduction Test", Part I, adopted April 04, 1984

5. RCC Study Number 797242; Determination of the Water Solubility of WACKER BS 1701

6. RCC Study Number 797275; Hydrolysis of WACKER BS 1701 at different pH values

7. The OECD Principles of Good Laboratory Practice, adopted by Council on 26th November 1997 [C(97)186/Final], Environment Directorate, Organisation for Economic Co-operation and Development, Paris 1998)

8. Thompson, W.R., Weil, C.S. (1952): On the construction of tables for moving average interpolation, Biometrics 8, 51 - 54

9. Distribution of the Final Report

Sponsor:	the original final report
IBACON:	one certified copy of the final report

Appendix

Table 1. Influence of Wacker BS 1701 on the mobility of *Daphnia magna*

Concentration of the test item [mg/L]	No. of *Daphnia* tested	No. of immobilized *Daphnia* after		% of immobilized *Daphnia* after	
		24 h	48 h	24 h	48 h
control	20	0	0	0	0
filtrate	20	0*	16	0	80
0.32	20	0	0	0	0
1.0	20	0	2	0	10
3.2	20	0	0	0	0
10	20	0*	7**	0	35
32	20	13**	20	65	100
100	20	20	20	100	100

* all *Daphnia* were trapped at the water surface but were not immobile
** all surviving *Daphnia* were trapped at the water surface

Table 2. Dissolved oxygen concentrations in the freshly prepared and old test media

Concentration of the test item [mg/L]	Oxygen [mg/L]			
	day 0 new	day 1 old	day 1 new	day 2 old
control	8.6	8.4	8.4	8.4
filtrate	8.0	8.5	7.9	8.6
0.32	8.6	8.6	8.6	8.4
1.0	8.6	8.5	8.6	8.5
3.2	8.5	8.6	8.5	8.5
10	8.5	8.7	8.4	8.4
32	8.6	8.7	8.4	8.6
100	8.6	8.7	8.6	*

* not determined since all *Daphnia* were dead after 24 hours test duration

Table 3. pH-values in the freshly prepared and old test media

Concentration of the test item [mg/L]	pH			
	day 0 new	day 1 old	day 1 new	day 2 old
control	7.7	7.6	7.7	7.8
filtrate	7.8	7.9	7.6	7.8
0.32	7.7	7.3	7.7	7.8
1.0	7.8	7.6	7.7	7.8
3.2	7.8	7.8	7.6	7.9
10	7.8	7.9	7.7	7.9
32	7.8	7.9	7.7	7.8
100	7.8	7.9	7.7	*

* not determined since all *Daphnia* were dead after 24 hours test duration

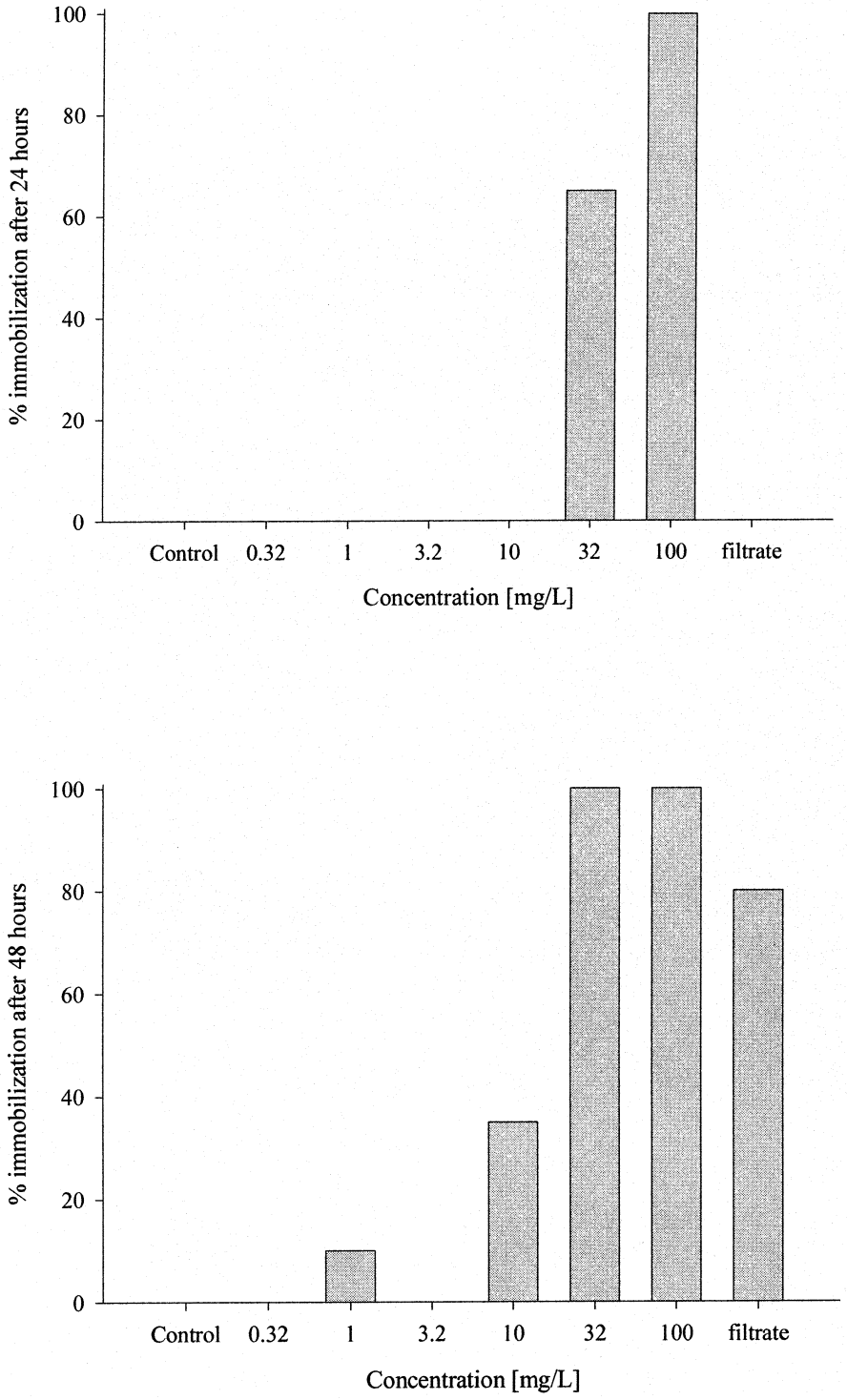


Figure 1. Percentage immobility of *Daphnia magna* at different concentrations of the test item after 24 and 48 hours test duration

Final Report

Determination of Wacker BS 1701 in Samples from Acute Toxicity to *Daphnia magna* in a 48-hour Immobilization Test

Study Director

Dr. Reiner Kiefer

Date

February 26, 2001

Testing Facility

Arbeitsgemeinschaft
GAB Biotechnologie GmbH &
IFU Umweltanalytik GmbH
Eutinger Str. 24
D-75223 Niefern-Öschelbronn
Germany

Sponsor

IBACON
Institut für Biologische Analytik
und Consulting GmbH
Arheilger Weg 17
D-64380 Roßdorf
Germany

Study Identification Code

Test substance: Wacker BS 1701

Study code of Testing Facility: 20011012/01-UW

Study code of Sponsor: 9541220

Statement of Confidentiality

This report contains confidential and proprietary information of IBACON which must not be disclosed to anyone except the employees of this company or to persons authorized by law or judicial judgement without the expressed and written approval of IBACON.

Statement of Compliance with the Principles of Good Laboratory Practice

The study described in this report was conducted in compliance with the most recent edition of:

- The Principles of Good Laboratory Practice (GLP), (Chemikaliengesetz, attachment 1, Federal Republic of Germany).
- The OECD Principles of Good Laboratory Practice.

The German requirements are based on the OECD Principles of Good Laboratory Practice which are accepted by regulatory authorities throughout the European Community, the United States of America (FDA and EPA) and Japan (MHW, MAFF and MITI) on the basis of intergovernmental agreements.

Head of testing facility
(Dr. Hans Eberhardt)

26 Feb 2001
Date / Signature

Study director
(Dr. Reiner Kiefer)

26 Feb 2001
Date / Signature

Statement of Quality Assurance Unit

Study code: 20011012/01-UW

Study title: Determination of Wacker BS 1701 in Samples from Acute Toxicity to *Daphnia magna* in a 48-hour Immobilization Test

Study plan, draft report and final report were audited by the Quality Assurance Unit. The experimental phase was audited as process audit. The dates are given below:

	Date of audit	Date of report
Study plan:	22/01/2001	22/01/2001
Experimental phase	08/09/2000	04/10/2000
Draft report:	26/02/2001	26/02/2001
Final report:	27/02/2001	27/02/2001

Quality assurance manager:
(Dr. Susanne Timmermann)

27 Feb 01 S. Timmermann
Date / Signature

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1 Performing Laboratory

The study was performed at the analytical laboratory of the testing facility:

IFU Umweltanalytik GmbH
Bleichstr. 19
D-75173 Pforzheim
Germany
Phone ++49-7231-9236-20
Fax ++49-7231-9236-66

2 Study Objective

The objective of the present study was the determination of the concentrations of Wacker BS 1701, determined by measuring the concentrations of silicon by ICP-AES, in the samples as listed below in table 1:

Table 1: List of samples

IFU-J.-No.	Sample No. Sponsor	Description	Day	Age	Date
2001-00196	D1	9541220 Kontrolle-1	0	0 h	09/01/01
2001-00197	D2	9541220 Konz.3 3.2 mg/L-1	0	0 h	09/01/01
2001-00198	D3	9541220 Konz.3 3.2 mg/L-2	0	0 h	09/01/01
2001-00199	D4	9541220 Konz.4 10 mg/L-1	0	0 h	09/01/01
2001-00200	D5	9541220 Konz.4 10 mg/L-2	0	0 h	09/01/01
2001-00201	D6	9541220 Konz.5 32 mg/L-1	0	0 h	09/01/01
2001-00202	D7	9541220 Konz.5 32 mg/L-2	0	0 h	09/01/01
2001-00203	D8	9541220 Konz.6 100 mg/L-	0	0 h	09/01/01
2001-00204	D9	9541220 Konz.6 100 mg/L-	0	0 h	09/01/01
2001-00205	D10	9541220 Kontrolle-1	1	24 h	10/01/01
2001-00206	D11	9541220 Konz.3 3.2 mg/L-1	1	24 h	10/01/01
2001-00207	D12	9541220 Konz.3 3.2 mg/L-2	1	24 h	10/01/01
2001-00208	D13	9541220 Konz.4 10 mg/L-1	1	24 h	10/01/01
2001-00209	D14	9541220 Konz.4 10 mg/L-2	1	24 h	10/01/01
2001-00210	D15	9541220 Konz.5 32 mg/L-1	1	24 h	10/01/01
2001-00211	D16	9541220 Konz.5 32 mg/L-2	1	24 h	10/01/01
2001-00212	D17	9541220 Konz.6 100 mg/L-	1	24 h	10/01/01
2001-00213	D18	9541220 Konz.6 100 mg/L-	1	24 h	10/01/01

2001-00214	D19	9541220 Konz.7 Filtrat von 100 mg/L-1	1	24 h	10/01/01
2001-00215	D20	9541220 Konz.7 Filtrat von 100 mg/L-2	1	24 h	10/01/01
2001-00216	D21	9541220 Kontrolle-1	1	0 h	10/01/01
2001-00217	D22	9541220 Konz.3 3.2 mg/L-1	1	0 h	10/01/01
2001-00218	D23	9541220 Konz.3 3.2 mg/L-2	1	0 h	10/01/01
2001-00219	D24	9541220 Konz.4 10 mg/L-1	1	0 h	10/01/01
2001-00220	D25	9541220 Konz.4 10 mg/L-2	1	0 h	10/01/01
2001-00221	D26	9541220 Konz.5 32 mg/L-1	1	0 h	10/01/01
2001-00222	D27	9541220 Konz.5 32 mg/L-2	1	0 h	10/01/01
2001-00223	D28	9541220 Konz.6 100 mg/L-	1	0 h	10/01/01
2001-00224	D29	9541220 Konz.6 100 mg/L-	1	0 h	10/01/01
2001-00225	D30	9541220 Kontrolle-1	2	24 h	11/01/01
2001-00226	D31	9541220 Konz.3 3.2 mg/L-1	2	24 h	11/01/01
2001-00227	D32	9541220 Konz.3 3.2 mg/L-2	2	24 h	11/01/01
2001-00228	D33	9541220 Konz.4 10 mg/L-1	2	24 h	11/01/01
2001-00229	D34	9541220 Konz.4 10 mg/L-2	2	24 h	11/01/01
2001-00230	D35	9541220 Konz.5 32 mg/L-1	2	24 h	11/01/01
2001-00231	D36	9541220 Konz.5 32 mg/L-2	2	24 h	11/01/01
2001-00232	D37	9541220 Konz.7 Filtrat von 100 mg/L-1	2	24 h	11/01/01
2001-00233	D38	9541220 Konz.7 Filtrat von 100 mg/L-2	2	24 h	11/01/01
2001-00278	D39	9541220 Konz.7 Filtrat von 100 mg/L-1	1	0 h	10/01/01
2001-00279	D40	9541220 Konz.7 Filtrat von 100 mg/L-2	1	0 h	10/01/01
2001-00306	A1	Stabil 0.3 A	-	-	09/01/01
2001-00307	A2	Stabil 0.3 B	-	-	09/01/01
2001-00308	A3	Stabil 3 A	-	-	09/01/01
2001-00309	A4	Stabil 3 B	-	-	09/01/01
2001-00310	A5	Stabil 100 A	-	-	09/01/01
2001-00311	A6	Stabil 100 B	-	-	09/01/01

3 Summary

In the proposed time schedule, it was not possible to establish a reproducable method to determine the concentrations of Wacker BS 1701 by either ICP-AES or graphite furnace AAS.

There are two main technical reasons responsible:

In the performance of the ICP-AES analysis, there appeared an organic and a water phase in the atomizing chamber after injecting the samples, resulting in significant memory effects. Using alternate solvents like acetone, ethyl acetate, or ethanol, respectively, and the technical equipment variation using a cross flow atomizer did not give better results.

Varying the analytical method by using the graphite furnace AAS did not give reproducable and acceptable results because of formation of silicon carbide in the graphite tube, resulting in significant memory effects in the course of the measurement.

4 Materials and Methods

4.1 Test Substance

Common Name:	Wacker BS 1701
IUPAC Name:	Triethoxy(2,4,4-trimethylpentyl)silan
CAS-Name:	Silane, triethoxy(2,4,4-trimethylpentyl)
CAS-Number	35435-21-3
GAB-Code:	20011012
Supplier:	Wacker-Chemie GmbH
Batch No.:	KH 02343
Active Ingredient:	Alkylalkoxysilane
Purity:	98.53 %
Aggregrate state at RT:	liquid
Colour:	colourless
Density (at 25 °C):	0.86 g/cm^3
Solubility:	insoluble in water
Expiry date:	November 2001
Storage conditions:	at 20 °C, in the dark

4.2 Reference Substance

Common name:	Silicon Standard Solution
IUPAC Name:	Ammoniumhexafluorosilikat
GAB code:	20011020
Charge:	80301644
Product No:	1.12310.0100
Silicium content:	1000 mg/l
Expiry date:	07/01
Certificate of analysis:	07/98
Supplier:	Merck

4.3 Procedure

4.3.1 Equipment

Normal laboratory glassware and instrumentation

ICP Spectroflame D, Spectro Analytical Systems

Perkin Elmer AAS 4100 with HGA 700

4.3.2 Reagents

Deionized water, prepared at testing facility

Dimethylformamide (DMF)

Ethanol

Acetone

Ethyl acetate

4.3.3 Preparation of Standard Solutions (ICP-AES)

Standard solutions were prepared by dilution of stock solutions of the reference substance (1000 mg/L, Merck) with deionized water. The nominal concentrations were 2.0 mg/L, 4.0 mg/L, 8.0 mg/L, and 16.0 mg/L, respectively. All standard solutions were prepared fresh on the day of test substance analysis.

4.3.4 Sample preparation (ICP-AES)

To determine the repeatability and the recovery of the analytical method, stock solutions were prepared by accurately weighing 100 mg of Wacker BS 1701 (test substance) into a 100 mL flask and mixing with N,N-Dimethylformamide and deionized water.

4.3.5 ICP-AES Analysis

At the beginning of the measuring sequence, a calibration curve was prepared. Five concentrations (blank, 2.0 mg/L, 4.0 mg/L, 8.0 mg/L, and 16.0 mg/L) of the reference substance covering the concentration range of the test substance solutions were injected (calibration data, see figure 1 in the appendix).

A typical ICP-AES scan of silicon at 251.611 nm is illustrated in figure 2.

4.3.6 Results of the ICP-AES Analysis

The analysis of test substance solutions to determine the repeatability and the recovery of the analytical method, respectively, did not give reproducable and acceptable results.

The reason for this observation was the appearing phase separation of the emulsion. In the atomizing chamber of the ICP-AES, there appeared an organic and a water phase, resulting in memory effects in the course of the measurement.

The alternate sample preparation by dissolving the test substance in acetone, ethyl acetate, or ethanol, respectively, and the technical equipment variation using a cross flow atomizer did not give better results.

4.3.7 Preparation of Standard Solutions (Graphite Furnace AAS)

As an alternate method to determine the silicon concentrations in the test samples, the determination by graphite furnace AAS was tested.
Standard solutions were prepared by dilution of stock solutions of the reference substance (1000 mg/L, Merck) with deionized water. The nominal concentrations were 100 µg/L, 200 µg/L, 300 µg/L, 400 µg/L, 500 µg/L, respectively. All standard solutions were prepared fresh on the day of test substance analysis.

4.3.8 Sample preparation (Graphite Furnace AAS)

To determine the repeatability and the recovery of the analytical method, solutions at two concentration levels were prepared by accurately weighing 103.4 mg (1) and 203.67 mg (2), respectively, of Wacker BS 1701 (test substance) into a 100 mL flask and mixing with ethanol and deionized water.

Using ethanol as solvent, the best results in forming a stable emulsion were obtained.

4.3.9 Graphite Furnace AAS

At the beginning of the measuring sequence, a calibration curve was prepared. Six concentrations (blank, 100 µg/L, 200 µg/L, 300 µg/L, 400 µg/L, and 500

µg/L) of the reference substance covering the concentration range of the test substance solutions were injected. The corresponding calibration curve is shown in figure 3.

4.3.10 Results of the Graphite Furnace AAS Analysis

The analysis of test substance solutions to determine the repeatability and the recovery of the analytical method, respectively, did not give reproducable and acceptable results.

The reason for this observation was the formation of silicon carbide in the graphite tube, resulting in memory effects in the course of the measurement.

5 Deviations from the Study Plan

The study was performed according to the study plan dated January 19, 2001, with the following deviations:

The determination of silicon was alternatively tested by graphite furnace AAS.
Reason for change: It was not possible to quantify the contents of Wacker BS 1701 by determination of silicon by ICP-AES.
Impact on study: None

6 Results and Discussion

Two analytical methods to quantify the concentrations of Wacker BS 1701 by determination of silicon were tested:

ICP-AES and graphite furnace AAS.

The tested methods were not successful because of phase separation of the samples in the atomizer chamber (ICP-AES) and the formation of silicon carbide in the graphite tube (graphite furnace AAS), respectively, and the resulting significant and not reproducable memory effects. Using alternate solvents like acetone, ethyl acetate, or ethanol, respectively, and the technical equipment variation using a cross flow atomizer did not give better results.

7 Archiving

For the periods demanded by the principles of GLP the following documents and materials will be archived:

- Study plan, raw data, comments of the sponsor on the draft report and one original signed copy of the final report.
- All documentation generated by the Quality Assurance Unit
- A sample of the test substance.

All documents and materials will be stored in the archives of Arbeitsgemeinschaft GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH. The premises for storing the documents and materials are settled according to the principles of Good Laboratory Practice in the organization of the testing facility.

8 Distribution

Study Plan:	Testing facility (1 x) Sponsor (1 x)
Final Report:	Testing facility (1 x) Sponsor (1 x)
Raw Data:	Testing facility (1 x)

9 Appendix

Standard [No]	Concentration [mg/l]	Peak Height [Intensity]
Blank	0	61,3
1	2	14032
2	4	29206
3	8	57638
4	16	115405

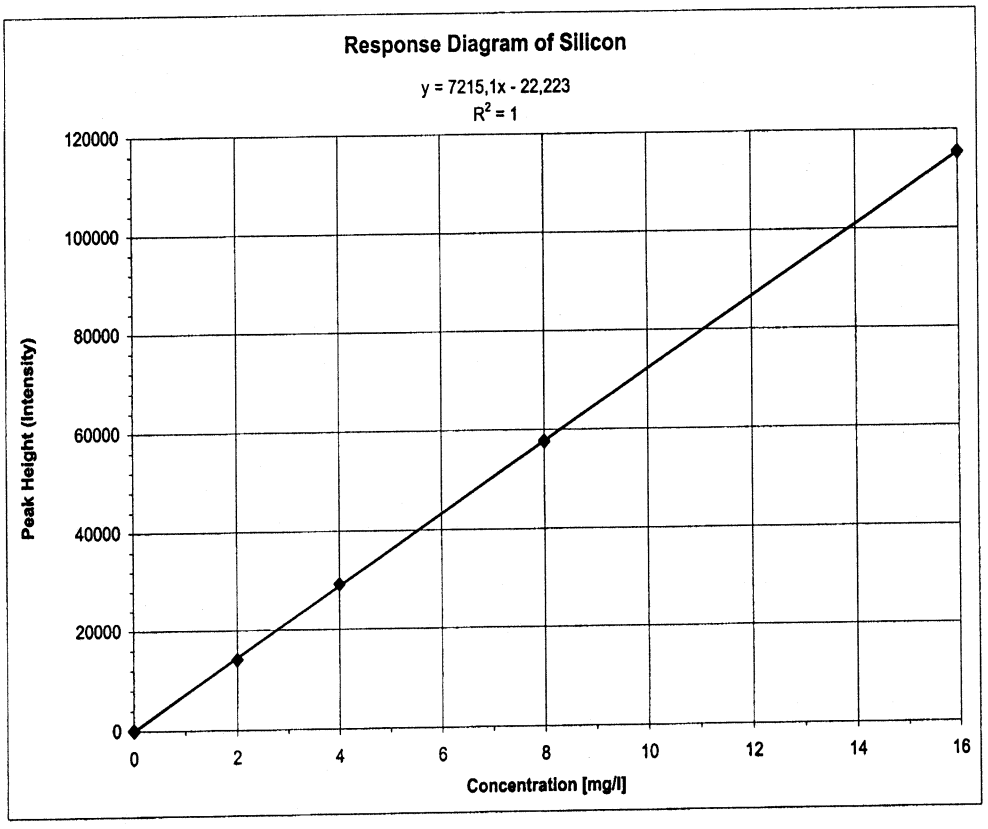

Figure 1: Calibration graph and detector response for analysis of silicon by ICP-AES

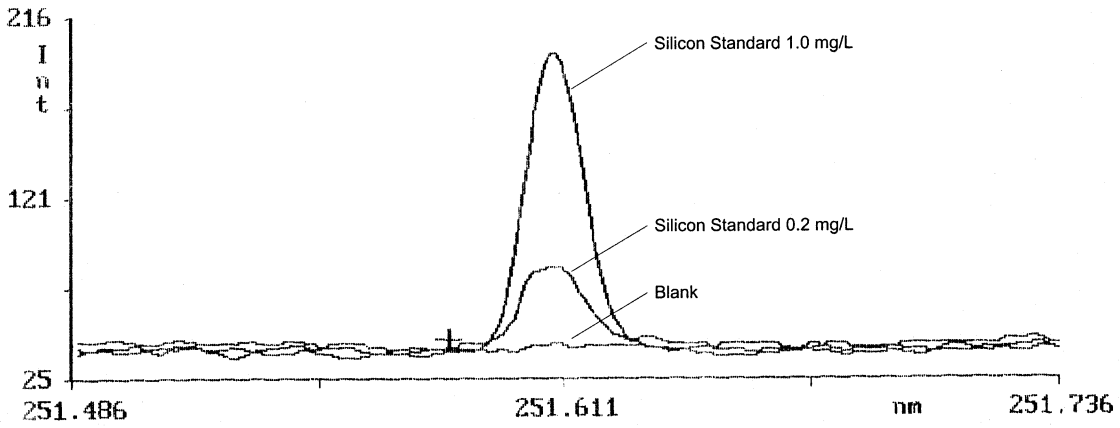

Figure 2: ICP-AES wavelength scans, silicon - calibration standards and blank - quantification wavelength 256.611 nm

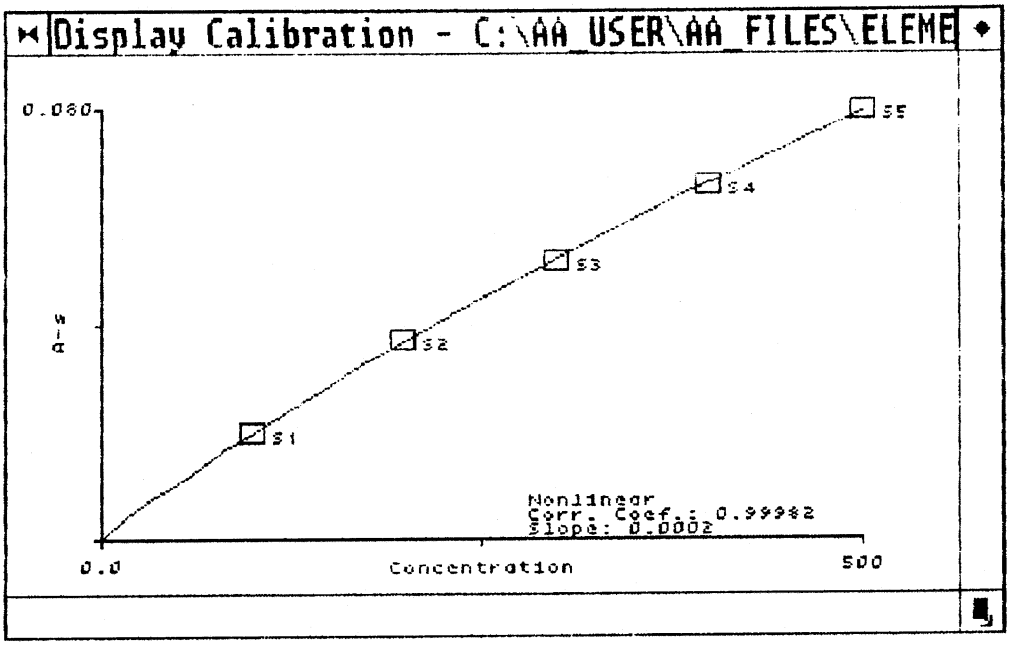

Figure 3: Graphite Furnace AAS calibration curve, silicon
x-axis: concentration [µg/L]; y-axis: peak area

Analysenzertifikat

MERCK

Kunde:

0000838080 D
IFU Umweltanalytik GmbH
-H.Vogel-

Bleichstr. 19
75173 Pforzheim

Ihr Ansprechpartner:
Holger Harnischfeg FLD/KMH
Telefon: *06151/722861*

Kundenbestellnummer: *0001063014/0006443495/H.Vogel / 1*
Kundenbestelldatum: *16.01.2001*
Kundenproduktnummer: *1.12310.0100*

Kundennummer: *184262*
Auftragsnummer: *11554310*
Lieferscheinnummer: *23705515*

Druckdatum: *17.01.2001*

1.12310.0100 Silicium-Standardlösung (sauer)
Ammoniumhexafluorosilikat in Wasser 1000 mg/l Si

Charge 80301644

	Chargenwerte	
Konzentration β (Si)	1000	mg/l

Bestimmungsmethode: *acidimetrische Titration.*
Methodengenauigkeit: *+/- 2 mg*

Freigabedatum: *16.07.1998*
mindestens verwendbar bis: *31.07.2001*

Wolfgang Gernand

Analytisches Labor

Dieses Dokument wurde maschinell erstellt und ist ohne Unterschrift gültig.

Merck KGaA 64271 Darmstadt Tel. (06151)72-0

Seite 1 von 1

Figure 4: Silicon reference substance - certificate of analysis -

Figure 5: GLP Certificate of testing facility

MINISTERIUM FÜR UMWELT UND VERKEHR
BADEN-WÜRTTEMBERG

Ministerium für Umwelt und Verkehr Baden-Württemberg, Pf. 10 34 39, 70029 Stuttgart

GLP-Bescheinigung

Bescheinigung

Hiermit wird bestätigt, daß die Prüfeinrichtung

in 75223 Niefern-Öschelbronn
Eutinger Straße 24

der Arbeitsgemeinschaft
GAB-Biotechnologie GmbH und
IFU-Umweltanalytik GmbH

am 13.04.2000

von der für die Überwachung zuständigen Behörde über die Einhaltung der Grundsätze der Guten Laborpraxis inspiziert worden ist.

Es wird hiermit bestätigt, daß folgende Prüfungen in dieser Prüfeinrichtung nach den Grundsätzen der Guten Laborpraxis durchgeführt werden.

Certificate

It is hereby certified that the test facility

in 75223 Niefern-Öschelbronn
Eutinger Straße 24

of Arbeitsgemeinschaft
GAB-Biotechnologie GmbH und
IFU-Umweltanalytik GmbH

on 13.04.2000

was inspected by the competent authority regarding compliance with the Principles of Good Laboratory Practice.

It is hereby certified that studies in this test facility are conducted in compliance with the Principles of Good Laboratory Practice.

Stuttgart, 20.07.2000

Kernerplatz 9 Telefax Zentral / Pressestelle
70182 Stuttgart (07 11) 1 26 - 28 81 / 28 80
S-Bahn: Haltestelle Hauptbahnhof
U1, U4, U9, U14, Bus 40, 42: Haltestelle Staatsgalerie

☎-Vermittlung: (07 11) 1 26 - 0
X400: s=poststelle, o=uvm, p=bwl, a=dbp, c=de
Internet-eMail: poststelle@uvm.bwl.de
Internet: http://www.uvm.baden-wuerttemberg.de

Hauptstätter Straße 67 Telefax
70178 Stuttgart (07 11) 1 26 - 10 99
S-Bahn: Haltestelle Stadtmitte
U1, U14, Bus 44: Haltestelle Österreichischer Platz

Prüfkategorie 1	**Prüfungen zur Bestimmung der physikalisch-chemischen Eigenschaften und Gehaltsbestimmungen**
Prüfkategorie 4	**Ökotoxikologische Prüfungen zur Bestimmung der Auswirkungen auf aquatische und terrestrische Organismen**
Prüfkategorie 5	**Prüfungen zum Verhalten im Boden, im Wasser und in der Luft; Prüfungen zur Bioakkumulation und zur Metabolisierung**
Prüfkategorie 6	**Prüfungen zur Bestimmung von Rückständen**
Prüfkategorie 7	**Prüfungen zur Bestimmung der Auswirkungen auf Mesokosmen und natürliche Ökosysteme**
Prüfkategorie 8	**Analytische Prüfungen an biologischen Materialien**

Dies entspricht den Kategorien der Allgemeinen Verwaltungsvorschrift zum Verfahren der behördlichen Überwachung der Einhaltung der Guten Laborpraxis (ChemVwV-GLP) vom 15. Mai 1997.

Dr. Albrecht

Stuttgart, den 20.07.2000